

P.C. 2108.91



PATENT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

In re application of)

LAWRENCE A. JOHNSON)

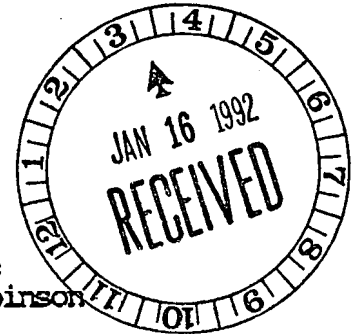
Method to Preselect the)
Sex of Offspring)

Serial No. 07/692,958)

Filed April 26, 1991)

Group Art Unit 188
Examiners J. Witz &
D. W. Robinson

[2108DLG]



DECLARATION UNDER 37 CFR 1.132

Duane L. Garner deposes and states:

1. That he considers himself to be an expert in the art of preparing mammalian sperm for artificial insemination as evidenced by the attached curriculum vitae;

2. That he has read and is familiar with the following publications authored by Johnson et al.:

- "Flow Sorting of X and Y Chromosome-Bearing Spermatozoa Into Two Populations," *Gamete Research* 16:1-9 (1987);
- "Flow Cytometry of X and Y Chromosome-Bearing Sperm for DNA Using an Improved Preparation Method and Staining With Hoechst 33342," *Gamete Research* 17:203-212 (1987);

■ "Flow Sorting of X and Y Chromosome-Bearing Mammalian Sperm:
Activation and Pronuclear Development of Sorted Bull, Boar, and
Ram Sperm Microinjected Into Hamster Oocytes," *Gamete*
Research 21:1-9 (1988);

3. That in his opinion the various treatments preparatory to staining the sperm cells in each of the publications referred to in (2) would in every case cause the tails and midsections of the sperm to become severed from the sperm heads and that the plasma and acrosomal membranes surrounding the resultant sperm heads would thereby be disrupted;

4. That the treatments referred to in (3) would render the sperm cells completely nonviable and would facilitate absorption of stain such as Hoechst 33342 by the disrupted sperm;

5. That, absent any knowledge he has of the subject matter of the above-identified application, he would have expected that any extended incubation of viable sperm at temperatures within the range of 30-39° C would be detrimental to the ultimate viability of the sperm and would not be a recommended procedure;

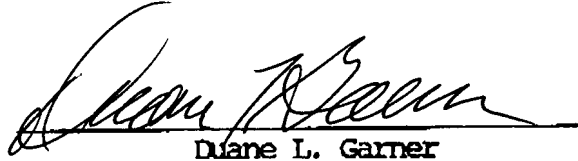
6. That in his opinion incubation at room temperature for a period of 3-5 hours would tend to decrease viability of sperm that was intended for use in artificial insemination;

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7. That it is his understanding that treatments accorded sperm for Artificial Insemination always include cooling as soon as practicable after ejaculation to begin the slowing of sperm metabolism and that in his experience in the area of sperm physiology and artificial insemination, one would never consider incubating sperm at 30-39° C for extended time periods, because it is contrary to the maintenance of sperm viability; and

8. That all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Executed December 20, 1991, 1991.


Duane L. Garner

State of Nevada

SS.

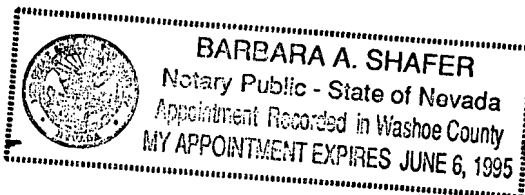
County of Clark

On this 20 day of December, in the year 1991, before me, Barbara A. Shafer/ Notary Public, personally appeared Duane L. Garner, personally known to me to be the person whose name is subscribed to this instrument, and acknowledged that he executed it.

WITNESS my hand and official seal:



- 3 -



DUANE L. GARNER - CURRICULUM VITAE

December 1991

PERSONAL

<u>Title and Address</u>	<u>Home Address</u>
Professor of Animal Science School of Veterinary Medicine (Mail Stop 202) University of Nevada Reno, Nevada 89557-0104 Telephone: 702/784-1357 FAX: 702/784-1375	2660 Everett Drive Reno, Nevada 89503 Telephone: 702/747-7746

<u>Date And Place Of Birth</u>	<u>Marital Status</u>
February 9, 1936 Madera, California	Married: June A. Garner Three children

Veteran: 24 months service - Veterinary Specialist, U.S. Army Medical Research Laboratory, Ft. Knox, Kentucky - Honorable Discharge

SCHOLASTIC TRAINING

B.S.	California State University, Fresno	1964
	Major: Animal Husbandry	
M.S.	Washington State University	1967
	Major: Animal Science	
Ph.D.	Washington State University	1969
	Major: Animal Sciences (Reproductive Physiology)	
	Minor: Biochemistry	
	NDEA Title IV Fellowship	
	Advisor: Dr. M.H. Ehlers	
Post-Doctoral	University of Illinois, NICHD	1969-
	Postdoctoral Research Fellow	1970
	Research Fellowship: (USPH HD 44,623)	
	Sponsor: Dr. G.W. Salisbury	

PROFESSIONAL APPOINTMENTS

Research Associate in Dairy Science (Reprod. Physiol.), Dept. of Dairy Science, University of Illinois	1970-1972
Assistant Professor, Associate Professor and Professor of Physiological Sciences, College of Vet. Medicine, Oklahoma State University	1972-1985
Guest Scientist, Lawrence Livermore National Laboratory, Sabbatical Leave from Oklahoma State University	1981
Professor and Chair, Department of Animal Science, College of Agriculture, University of Nevada, Reno	1985-1991
Professor, School of Veterinary Medicine, College of Agriculture, University of Nevada, Reno	1991-present

PROFESSIONAL AND LEARNED SOCIETIES

American Association for the Advancement of Science	Alpha Zeta
American Society of Andrology	Sigma Xi
American Society of Animal Science	Phi Kappa Phi
Society for the Study of Reproduction	Phi Zeta
American Society for Cell Biology	Gamma Sigma
Society for Analytical Cytology	Delta
American Fertility Society	

BACKGROUND AND PROFESSIONAL ACADEMIC EXPERIENCE

BACKGROUND: Agricultural background acquired on parents' cattle and grain ranch in the San Joaquin Valley in California. During his high school years, Dr. Garner was a member of the state champion Future Farmers of America parliamentary procedure team, the livestock judging team and served as a regional as well as a chapter officer. He owned and managed a flock of Hampshire sheep for which he was awarded the Bank of America trophy for the best F.F.A. sheep project in the state. With this project Dr. Garner won 26 championships including four at the California State Fair and 13 showmanship contests including the F.F.A. Division at the state fair. He gained additional practical experience while employed as Student Shepherd at California State University, Fresno. Dr. Garner also gained experience as manager of a show flock for a purebred sheep breeder and has exhibited animals nationwide including the Cow Palace and Chicago International Livestock Exhibitions. Other experiences include 2 years as a veterinary specialist while fulfilling military obligation and 3 years as a USDA processed products inspector (during undergraduate years).

TEACHING: Dr. Garner gained initial teaching experience while assisting in laboratory sections of a farm accounting class at California State University, Fresno. Additional training was gained as a teaching assistant in the laboratory sections of an undergraduate course in reproductive physiology at Washington State University. While at Washington State and the University of Illinois, Dr. Garner gave several lectures on reproduction in farm animals. At Oklahoma State University and the University of Nevada, Reno, Dr. Garner had the following teaching responsibilities:

1. Instructor for PHSIO 4113. Anatomy & Physiology of Farm Animals. Fall and Spring 1972-73.
2. Instructor for PHSIO 6570. Physiology Seminar, Reproductive Physiology. Fall 1973, 1974, 1975, 1976, 1977, 1978, 1979, 1980 and Spring 1977, 1982.
3. Instructor for PHSIO 3004. Introductory Physiology. Fall 1973 (one-half semester) and Instructor for two laboratory sections, Fall 1983. Instructor, Spring 1975.
4. Instructor for PHSIO 3125 and 4223. Mammalian Physiology. Spring 1976, 1977, 1978 (Sections on Reproductive Physiology and Neurology).
5. Instructor for PHSIO 6223. Advanced Physiology of Reproduction. Spring 1974, 1976, 1980.

6. Instructor for PHSIO 5113. Basic Reproductive Physiology. Fall 1976, 1977, 1978, 1979, 1980, 1984, 1985.
7. Instructor for PHSIO 5253. Veterinary Endocrinology and Reproduction. Fall 1979, 1980, Spring 1982, 1983, 1984, 1985.
8. Instructor for ASc 791. Special Topics - Andrology. Spring 1987.
9. Instructor for ASc 782b. Advanced Topics in Reproduction-Gametes, Fall 1989.

In addition to the above teaching responsibilities, Dr. Garner has guest lectured and participated in other courses. At the University of Nevada, Reno Dr. Garner also taught ASc 790-Seminar, Spring 1986, 1987, 1988, 1989, 1990, 1991 and the last half of ASc 325 Animal Genetics, Fall 1988.

ADVISING: As a member of the University Pre-medical Advisory Committee, Dr. Garner advised some 45 undergraduate students, mainly concerning preparation for entrance into medical or dental schools. 1978-80.

ADMINISTRATIVE EXPERIENCE

Dr. Garner has served as Acting Director of Research, College of Veterinary Medicine, Oklahoma State University, and has served on several occasions as Acting Department Head for the Department of Physiological Sciences. From 1985-1991, he served as Chair of the Department of Animal Science at the University of Nevada, Reno. This department provided instructional programs in residence and to clientele throughout Nevada using extension programs. His administrative duties included management of a department consisting of 10 full-time faculty, 9 classified staff and numerous student employees. During this period the department operated the Animal Research and Extension Center (A 994 acre research field laboratory with a meat science laboratory and a modern dairy) and Equestrian Center. The department utilized approximately 300 cattle, 350 sheep and several horses. The departmental programs and facilities operated on a yearly budget of approximately \$350,000 and provided programs for about 25 undergraduate and 10 graduate students. The animal science program was merged with the School of Veterinary Medicine on July 1, 1991.

OTHER RESPONSIBILITIES

Dr. Garner has served on numerous committees including the Library Committee for the OSU College of Veterinary Medicine (Chairman), the College Curriculum Committee, the College Research Advisory Committee, the College Ad Hoc committee on Faculty Development and the department Graduate Education and Research Committee (Chairman, 3X), The Curriculum and Schedule Committee, and the Promotion and Tenure Committee (Chairman, 1X) for the Department of Physiological Sciences. He served on the Departmental Search committees for a Pharmacology and Cell Biology Faculty Position and an Anatomy Faculty Position. Dr. Garner served on a university-wide committee for improvement of faculty rewards at Oklahoma State. At UNR, Dr. Garner served on the College of Agriculture Personnel committee, the College Bylaws Committee, and chaired the Ad Hoc committee on Faculty Expectations and the Search Committee for the Associate Director of the Nevada Agricultural Experiment Station chairman. He served on a Search Committee for the Department of Biology. He served on the Sabbatical Leave

Committee and currently serves on the Institutional Laboratory Animal Care and Use Committee and the Academic Appeals Subcommittee.

Dr. Garner has directed programs of one doctoral, five masters level graduate students and currently advises one doctoral program student. Also, he has served on the advisory committee for seven masters and six doctoral level graduate students. Dr. Garner served on the Editorial Board of *Theriogenology - An International Journal of Animal Reproduction* (1976-1989), a consultant to the Agri-Practice Editorial Board, and as a reviewer for *Biology of Reproduction*, *Biochemistry*, *Cytometry*, *Gamete Research*, *Molecular Reproduction and Development*, the *Journal of Animal Science*, the *Journal of Andrology*, *Anatomia-Histologia-Embryologia-Journal of Veterinary Medicine*, the *Journal of Biological Physics*, *Journal Reproduction in Fertility*, and *Toxicology and Applied Pharmacology*.

OFFICE AND COMMITTEE ASSIGNMENTS HELD IN PROFESSIONAL AND HONORARY SOCIETIES

Chairman, Research Report Session on the Testis, annual Meeting of the Society for the Study of Reproduction, Athens, GA, 1973.

Member, Membership Committee, American Society of Andrology, 1975.

Listed, American Men and Women of Science: 1976, 1978, 1988.

Member, Student Affairs committee, American Society of Andrology, 1982-1984.

Member, Nominating Committee, Society for the Study of Reproduction, 1982.

Co-chairman, Research Report Session on Sperm Physiology, Annual Meeting of the Society for the Study of Reproduction, Madison, WI, 1982.

Delegate, OSU Representative at the 1983 Annual Meeting of Sigma Xi, Toronto, Canada, October 29-31, 1983.

Listed, Who's Who in Frontier Science and Technology, 1984.

President, Oklahoma State University Chapter of Sigma Xi, 1984-1985.

Chairman, Nominating Committee, American Society of Andrology 1989-1990.

Chairman, Western Section American Society of Animal Science Department Heads, 1988-1990.

Secretary-Treasurer, Western Section American Society of Animal Science, 1989-1990.

Member, Nominating Committee, American Society of Andrology 1990-1991.

President-Elect, Western Section American Society of Animal Science, 1990-1991.

President, Western Section American Society of Animal Science, 1991-1992.

PARTICIPATION IN TECHNICAL CONFERENCES, WORKSHOPS, ETC.

1. Harold C. Mack Symposium on "Biology of Mammalian Fertilization and Implantation," Wayne State University, Detroit, MI, 1970.
2. Gordon Research Conferences at Holderness School, Plymouth, NH.
 - a. "Fertilization Processes on Man and Mammals," 1974.
 - b. "Mammalian Genital Tract Secretions," 1977. (discussant)
 - c. "Fertilization and Activation of Development," 1977.
3. Mott Center Conference on "The Human Semen and Fertility Regulation in the Male," Wayne State University, Detroit, MI. 1975.

4. Special Invitations:
Speaker, Washington State University Sheep Day, 1967.
Speaker, University of Illinois Sheep Day, 1971.
Speaker, Oklahoma State University Sheep Day, 1974.
Collaborator, USDA, Agricultural Research Service, 1974.
Speaker, Fall Conference for Society of
Theriogenology, 1978.
5. Ad Hoc Consultant: NIH Population Research Committee, Program
Project Review Committee (1975); Population Training Grant Review
Committee (1976); the National Science Foundation (1978) and the
Binational Agricultural Research and Development Fund (1983, 1984).
6. Discussion Leader: "Sperm Fertilizing Ability," Eleventh Annual Meeting,
Society for the Study of Reproduction, Southern Illinois University,
Carbondale, IL, 1978.
7. MASUA Honor Lecturer: Dr. Garner was selected as a Mid-American
State Universities Association (MASUA) Honor Lecturer for 1978-79.
8. NIH Workshop on the "Testis," Houston, TX, 1979.
9. NIH Workshop on the Immunological Aspects of Infertility and Fertility
Regulation," St. Louis, MO. 1979.
10. Special Reviewer: NIH Reproductive Biology Special Study Section,
Grant Application Review, Houston, TX, 1980.
11. NIH Workshop on "Animal Fertilization and Development *In Vitro*: State
of the Art," National Institute of Child Health and Human
Development, Bethesda, MD, 1980.
12. NIH Workshop on "The Role of Proteins and Peptides in Control of
Reproduction", Bethesda, MD, 1982.
13. Special Reviewer: NIH Reproductive Biology Study Section, Review of
grant applications with the Study Section, Bethesda, MD, February,
1982.
14. Consultant: Lawrence Livermore National Laboratory, Biomedical
Science Division, Livermore, CA, 1982-85.
15. Conference on "Prospects of Sexing Mammalian Sperm," Denver, CO,
1982.
16. Member: Research Committee, National Association of Animal Breeders,
Columbus, MO, 1982-1987.
17. Consultant: American Cyanamid Co., Agricultural Research Division,
Princeton, NJ, 1983.
18. Co-arranger: "Control of Mammalian Sex Ratio at Birth," American
Association for the Advancement of Science, Session on Biological
Sciences, AAAS Meeting, Detroit, MI, 1983.
19. Special reviewer: NIH Reproductive Biology Study Section, review of
grant applications with the Study Section, Bethesda, MD, February 7-
10, 1984.
20. Member: Research Advisory Committee, Oklahoma City Zoo, OK, 1983-
84.
21. Co-recipient: Merck Foundation Animal Health Education Grant to
support College of Veterinary Medicine Visiting Scientists, Short
Course and Lecture Series, 1984-85.
22. Reviewer: United States-Israel BARD Fund Grants, 1984-85.
23. Reviewer: NIH Human Embryology and Development Special Study
Section, Grant Application Review, Washington D.C., 1984.
24. Reviewer: USDA Competitive Grants Program, 1985, 1988.
25. Member: NIH Reproductive Biology Study Section, 1986-1990.
26. Consultant: Select Sires, Plain City, OH, 1987.
27. Consultant: CYTOGAM, Chandler, AZ, 1987-1990.

28. Member, Research Liaison Committee, U.S. Sheep Experiment Station, Dubois, ID, 1988-90.
29. Reviewer: NIH Human Embryology and Development Special Study Section, Grant Application Review, Washington D.C., 1989.
30. Reviewer: National Institutes of Health Reviewers Reserve, 1990-1994.
31. Reviewer: March of Dimes Birth Defects Foundation, Grants Administration, White Plains, NY, 1990.
32. Reviewer: National Science Foundation, Cellular Biosciences Developmental Biology. 1990.
33. Reviewer: NIH Reproductive Biology Study Section, 1991.
34. Reviewer: Panel Member, USDA National Research Initiative Competitive Research Grants Program, Reproductive Biology of Animals, 1991.

RESEARCH

Dr. Garner began his research career as a Research Assistant at Washington State University. Since completing his Ph.D. in 1969, Dr. Garner has authored or co-authored fifty-three scientific publications and forty-nine abstracts. He has been senior author on twenty-five publications, including chapters on "Spermatozoa" in Reproduction in Farm Animals (4th, 5th and 6th editions) and on "Artificial Insemination" in Reproduction in Domestic Animals (4th ed). He has made twenty-four presentations at national scientific meetings and has delivered thirty-five invited extramural lectures.

PRINCIPAL INVESTIGATOR FOR RESEARCH PROJECTS:

1. The Physiology and Biochemistry of Mammalian sperm proteinases and their Natural Inhibitors (Illinois Hatch 35-339). \$20,500. 1971-1972.
2. Enzymology of Mammalian Sperm Acrosomes (USPH HD 07481- 01-06). \$187,077. 1972-1976.
3. Electroimmunoassay of Porcine Acrosin (USDA Coop. Agreement): Agricultural research Service, AP&GI, No. 12 - 14-7001-114. \$9,500. 1975--1980.
4. Biochemical Assessment of Fertilizing Capacity (National Association of Animal Breeders). \$15,000. 1976-1980.
5. Immunoabsorbent Isolation of Specific Spermatozoal Antigens for Use as Anti-fertility Immunogens (PARFR 99N); Program for Research on Fertility Regulation (USAID, Northwestern University). \$30,233. 1978.
6. PZ-Peptidases of Mammalian Spermatozoa and Testis (USPH 12477 01-04. \$216,822, 1979-1983.
7. Flow Cytometric Verification of the Relative Proportions of X- and Y-chromosome-Bearing sperm in bull and boar semen (USDA Coop. Agreement); Agricultural Research Service, Animal Science Institute, No. 12-13-7001-114 and 58-32U4-1- 343. \$50,000. 1981.
8. Fluorescent Staining Technology for *in vitro* Fertility Testing Procedures in Swine and Cattle Semen (USDA Coop. Agreement); Agricultural Research Service, Animal Science Institute, No. 58-7B30-3-510. \$4,000. 1983-1985.
9. Automated Assessment of Bovine Sperm Fertilizing Potential; USDA Competitive Research Grants Prog., 85-CGCG-1-1852. \$222,000. 1985-1989.
10. Automated Assessment of Feedstuff Toxicity on Reproduction in Rams, Hatch 313, Nevada Agricultural Experiment Station. Intramural. 1986-1989.
11. Gossypol Toxicity in Dairy Cattle Fed Whole Cottonseed, Hatch 317, Nevada Agricultural Experiment Station. Intramural. 1989-1992.

12. Augmentation of Sperm Transport in the Cow, National Association of Animal Breeders. \$10,000. 1989-1991.
13. Automated Assessment of Bovine Sperm Fertilizing Potential; USDA Competitive Research Grants Prog. 89-37240-4736, Renewal. \$123,000. 1989-1992.

COINVESTIGATOR FOR RESEARCH PROJECTS:

1. Metalloproteases of Mammalian Spermatozoa, B.A. Lessley, Principal Investigator (USPH 18828-01-02). \$142,938. 1984-1986.
2. Technics in Electrical Semen Collection in Birds, K.M. Betzen, W.S. Newcomer, D.L. Garner and L.L. Rolf (OSU College of Veterinary Medicine seed Grant, \$500). 1984-1985.
3. Effects of Advanced Uterine Development on Blastocyst Development and Survival, R.D. Geisert, Principal Investigator, USDA Competitive Grants Program. 1986.
4. Purchase of a Flow Cytometer, Biological Instrumentation Program, National Science Foundation, Doug Redelman, Principal Investigator. \$80,000. 1985.
5. Automated Assessment of Somatic Cell Populations in Holstein Milk, Hatch 312, Nevada Agricultural Experiment Station. Intramural. 1986-1987.

SABBATICAL LEAVE: Developed research expertise in flow cytometric analysis of spermatozoa while at the Lawrence Livermore National Laboratory (LLNL), Livermore, CA. Project involved development of flow cytometric methods to quantify mutagen-induced changes in sperm head shape (January-June 1981). A second six-month research assignment at LLNL to apply flow cytometric techniques to the quantification of the X- and Y-chromosome-bearing spermatozoa of bovine and porcine semen (July-December 1981). This project was supported by the U.S. Department of Agriculture.

JOURNAL PUBLICATIONS:

1. **Garner, D.L.**, and M.H. Ehlers. Effect of storage at 5 C on the disc electrophoretic patterns of ovine and bovine seminal proteins. *J. Reprod. Fertil.* 27:43-52. 1971.
2. **Garner, D.L.**, G.W. Salisbury and C.N. Graves. Electrophoretic fractionation of bovine acrosomal proteins and proteinase. *Biol. Reprod.* 4:93-100. 1971.
3. Dzuik, P.J., A.R. Ellicott, S.K. Webel, **D.L. Garner** and B.B. Doane. Conception rate in ewes mated or inseminated at an appointed time. *Am. J. Vet. Res.* 33:1589-1592. 1972.
4. **Garner, D.L.** and R.F. Cullison. Partial purification by affinity chromatography of bovine acrosin. *J. Chromatog.* 92:445-449. 1974.
5. **Garner, D.L.**, M.P. Easton, M.E. Munson and M.A. Doane. Immunofluorescent localization of bovine acrosin. *J. Exp. Zool.* 92:127-131. 1975.
6. **Garner, D.L.** Improved zymographic analysis of bovine acrosin. *Anal. Biochem.* 67:688-694. 1975.
7. Johnson, L.A., V.G. Pursel, N. Chaney and **D.L. Garner**. Comparison of several extraction procedures for boar spermatozoal acrosin. *Biol. Reprod.* 5:79-83. 1976.
8. **Garner, D.L.** and M.P. Easton. Immunofluorescent localization of acrosin in mammalian spermatozoa. *J. Exp. Zool.* 200:157-162. 1977.

9. **Garner, D.L.**, S.A. Reamer, L.A. Johnson and B.A. Lessley. Failure of immunoperoxidase staining to detect acrosin in the equatorial segment of spermatozoa. *J. Exp. Zool.* 201:309-315. 1977.
10. Lessley, B.A. and **D.L. Garner**. Isolation of motile spermatozoa by density gradient centrifugation in Percoll. *Gamete Res.* 7:49-61. 1983.
11. **Garner, D.L.**, B.L. Gledhill, D. Pinkel, S. Lake, D. Stephenson, M.A. Van Dilla and L.A. Johnson. Quantification of the X- and Y-chromosome-bearing spermatozoa of domestic animals by flow cytometry. *Biol. Reprod.* 28:312-321. 1983.
12. Lessley, B.A. and **D.L. Garner**. Distribution of Pz-peptidase in bovine epididymal and ejaculated semen. *Biol. Reprod.* 28:447-459. 1983.
13. Johnson, L.A., **D.L. Garner**, A.J. Truitt-Gibert and B.A. Lessley. Immunocytochemical localization of acrosin on the acrosomal membranes and acrosomal matrix of porcine spermatozoa. *J. Androl.* 4:222-229. 1983.
14. Amir, D., B.L. Gledhill, **D.L. Garner**, J.C. Nicolle, and A. Tadmor. Spermatogenic, epididymal, and spermatozoal damage induced by a peritesticular injection of ethylene dibromide to rams. *Animal Reprod. Sci.* 6:35-50. 1983.
15. Beal, W.E., L.M. White and **D.L. Garner**. Sex ratio following insemination of bovine spermatozoa isolated using a bovine serum albumin gradient. *J. Animal Sci.* 58:1432-1436. 1984.
16. Lessley, B.A. and **D.L. Garner**. Identification and preliminary characterization of two distinct bovine seminal Pz-peptidases. *Biol. Reprod.* 31:353-360. 1984.
17. **Garner, D.L.**, L.A. Johnson, S. Lake, N. Chaney, D. Stephenson, D. Pinkel and B.L. Gledhill. Morphological and ultrastructural characterization of mammalian spermatozoa processed for flow cytometric DNA analyses. *Gamete Res.* 10:339-351. 1984.
18. Pinkel, D., **D.L. Garner**, B.L. Gledhill, S. Lake, S. Stephenson and L.A. Johnson. Flow cytometric determination of the proportions of X- and Y-chromosome-bearing sperm in samples of purportedly separated bull sperm. *J. Animal Sci.* 60:1303-1307. 1985.
19. Lessley, B.A. and **D.L. Garner**. Identification and distribution of Pz-peptidases A and B in human semen. *J. Androl.* 6:372-378. 1985.
20. **Garner, D.L.**, D. Pinkel, L.A. Johnson and M.M. Pace. Assessment of spermatozoal function using dual fluorescent staining and flow cytometric analyses. *Biol. Reprod.* 34:127-138. 1986.
21. **Garner, D.L.**, L.A. Johnson and C.H. Allen. Fluorometric evaluation of cryopreserved bovine spermatozoa extended in egg yolk and milk. *Theriogenology* 30:369-378. 1988.
22. Redelman, D., S. Butler, J.D. Robison, and **D.L. Garner**. Identification of inflammatory cells in bovine milk by flow cytometry. *Cytometry* 9:463-468. 1988.
23. Ericsson, S.A., **D.L. Garner**, D. Redelman and K. Ahmad. Assessment of the viability and fertilizing potential of cryopreserved bovine spermatozoa using dual fluorescent staining and two flow cytometric systems. *Gamete Res.* 22:355-368. 1989.
24. Ericsson, S.A., **D.L. Garner**, L.A. Johnson, D. Redelman and K. Ahmad. Flow cytometric evaluation of cryopreserved bovine spermatozoa processed using a new antibiotic combination. *Theriogenology* 33:1211-1220. 1990.
25. Lessley, B.A., and **D.L. Garner**. Purification and initial characterization of Pz-peptidase B, an neutral metalloendopeptidase from bovine spermatozoa. *Biol. Reprod.* 43:643-658. 1990.

26. Kramer, R.Y., **D.L. Garner**, S.A. Ericsson, D. Redelman and T.W. Downing. The effect of cottonseed components on testicular development in pubescent rams. *Vet. Hum. Toxicol.* 33:11-19. 1991.
27. Downing, T.W., **D.L. Garner**, S.A. Ericsson and D. Redelman. Effects of fluorescent stains on the metabolism of cryopreserved bovine sperm. *J. Histochem. Cytochem.* 39:485-489. 1991.
28. Downing, T.W., **D.L. Garner**, S.A. Ericsson and G.C.J. Fernandez. Alteration of sperm metabolism by the addition of cytoplasmic droplets. *Arch. Androl.* (in press) 1991.
29. Sternes, K.L., B.K. Vig and **D.L. Garner**. Cytogenic variability and kinetochore proteins: comparison among populations derived from single-cell cultures. *Cancer Genet. Cytogenet* 56:000-000. 1991.

BOOK CHAPTERS:

1. **Garner, D.L.** and E.S.E. Hafez. Spermatozoa. In: E.S.E. Hafez (ed.), *Reproduction in Farm Animals*, 4th ed., Chapter 9, pp. 167-188. Lea and Febiger, Philadelphia, PA. 1979.
2. Gledhill, B.L., D. Pinkel, **D.L. Garner** and M.A. Van Dilla. Identifying X- and Y-chromosome bearing sperm by DNA content: Retrospective perspectives and prospective opinions. In: R.P. Amann and G.E. Siedel (eds.), *Prospects of Sexing Mammalian Sperm*, Colorado Associated University Press, Boulder, CO, 1982. pp. 177-191.
3. **Garner, D.L.** and E.S.E. Hafez. Spermatozoa and Seminal Plasma. In: E.S.E. Hafez (ed.), *Reproduction in Farm Animals*, 5th ed., Chapter 9, pp. 189-209. Lea and Febiger, Philadelphia, PA. 1986.
4. **Garner, D.L.** Artificial Insemination. In: P.T. Cupps (ed.), *Reproduction in Domestic Animals*, 4th ed. pp. 251-278, Academic Press, NY. 1991.
5. **Garner, D.L.** and E.S.E. Hafez. Spermatozoa and Seminal Plasma. In: E.S.E. Hafez (ed.), *Reproduction in Farm Animals*, 6th ed., Chapter 7, (in press). Lea and Febiger, Philadelphia, PA. 1991.

OTHER PUBLICATIONS:

1. **Garner, D.L.** The hyaluronidase activity of ram semen and its relationship to other seminal constituents. M. S. Thesis. Washington State University. 1967.
2. **Garner, D.L.** Fertility problems in sheep. Proceedings Northwest Lamb-Carcass Show and Washington State University Sheep Day. 1967.
3. **Garner, D.L.** Disc electrophoretic and zymographic studies of certain seminal proteins in the ram and bull. Ph.D. dissertation. Washington State University. 1969.
4. **Garner, D.L.** and C.N. Graves. Activity of proteolytic enzymes in spermatozoa. *Illinois Research* 13:14-15. 1971.
5. **Garner, D.L.** Biochemical assessment of fertilizing capacity. *Advanced Animal Breeder*, Vol. XXVI (4), p. 13. May. 1978.
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24. Ericsson, S.A.*, **D.L. Garner**, T.W. Downing and C.E. Marshall. Tri-functional cytometric analyses of fertility-tested cryopreserved bovine spermatozoa. American Society of Andrology, Montreal, Canada. Abst. 98 April 29, 1991.
25. **D.L. Garner**, S.A. Ericsson and D. Redelman*. Indo-1 labeling to discriminate motile sperm by flow cytometry. International Society for Analytical Cytology, Bergen, Norway, August 25-30, 1991.

INVITED EXTRAMURAL LECTURES AND SEMINARS BY DUANE L. GARNER

1. Molecular Forms of Spermatozoan Proteinase, Oklahoma State University, Stillwater, OK, April 20, 1972.
2. Acrosomal Proteinases in Rabbit, Ram and Bull Spermatozoa, U.S. Meat Animals Research Center, Clay Center, NE, April 26, 1972.
3. Molecular Forms of Spermatozoan Proteinase, The Johns Hopkins University, Baltimore, MD, May 3, 1972.
4. Augmentation of Accelerated Lambing Programs Using Ovulation Control, Oklahoma State University, Stillwater, OK, May 11, 1972.
5. Bovine Acrosomal Proteinase, USDA Agricultural Research Center, Animal Physiology and Genetics Institute, Beltsville, MD, February 28, 1974.
6. Synchronization, Insemination and Acrosomal Proteinase, University of California, Davis, CA, January 6, 1976.
7. How Sperm Work, Clinical Endocrine Conference, Veterans' Administration Hospital, Omaha, NE, October 28, 1976.
8. Spermatogenesis in the Mammal, University of Nebraska Medical Center, Omaha, NE, October 29, 1976.
9. Acrosomal Proteinases, University of Nebraska Medical Center, Omaha, NE, October 29, 1976.
10. Immunocytochemical Localization of Acrosin, A Symposium on Animal Agriculture, University of Illinois, Urbana, IL, May 24, 1978.
11. The Bovine Spermatozoan: Saga of a Single Cell, Fall Conference, Society for Theriogenology, Oklahoma City, OK, September 22, 1978.

12. The Bovine Spermatozoan: Saga of a Single Cell, Department of Biochemistry, Oklahoma State University, Stillwater, OK, October 13, 1978.
13. Assessment of Fertilizing Capacity, Animal Reproduction Laboratory, Department of Physiology and Biophysics, Colorado State University, Ft. Collins, CO, March 26, 1979.
14. Mammalian Sperm Proteinases, Department of Clinical Sciences, Colorado State University, Ft. Collins, CO, March 27, 1979.
15. Assessment of Fertilizing Capacity, Genetics Program Seminar, Oklahoma State University, Stillwater, OK, April 3, 1979.
16. The Bovine Spermatozoan: Saga of a Single Cell, University of California, Davis, CA, April 23, 1979.
17. Acrosin in Mammalian Spermatozoa, U.S. Meat Animal Research Center, Clay Center, NE, August 16, 1979.
18. Progress Report: Verification of X-Y Sperm Ratio, U.S.D.A. Agricultural Research Center, Animal Sciences Institute, Beltsville, MD, October 14, 1981.
19. Flow Cytometric Determination of X-Y Sperm Ratios, Meeting of the Oklahoma Society of Physiologists, Oklahoma State University, Stillwater, OK, June 11, 1982.
20. Flow Cytometric Determination of X-Y Sperm Ratios, Department of Biochemistry, Oklahoma State University, Stillwater, OK, October 22, 1982.
21. Flow Cytometric Determination of X-Y Sperm Ratios, Department of Obstetrics and Gynecology, Grand Rounds, University of Louisville School of Medicine, Louisville, KY, October 27, 1982.
22. Automated Assessment of Sperm Morphology, Endocrine Seminar, Department of Obstetrics and Gynecology, University of Louisville School of Medicine, Louisville, KY, October 17, 1982.
23. Flow Cytometric Determination of X-Y Sperm Ratios, Department of Biochemistry, Texas Tech University Health Sciences, Lubbock, TX, February 14, 1983.
24. Fertilization - Initiation of Development, Department of Biochemistry, Texas Tech University Health Sciences, Lubbock, TX, February 14, 1983.
25. Flow Cytometric Determination of X-Y Sperm Ratios in Mammalian Semen, Reproduction Laboratory, ARS, USDA, Beltsville Agricultural Research Center, Beltsville, MD, March 9, 1983.
26. Identification and Isolation of Sex-Specific Sperm in Major Domestic Mammalian Species, Agricultural Research Division, American Cyanamid Company, Princeton, NJ, March 11, 1983.
27. An Overview of Separation of X- and Y-Spermatozoa - Sexing, National Association of Animal Breeders, 10th Technical Conference, Milwaukee, WI, April 14, 1984.
28. *In Vitro* Methods for Estimating Fertilizing Capacity of Sperm Cells, 10th International Congress on Animal Reproduction and Artificial Insemination, Urbana, IL, June 10-14, 1984.
29. Assessment of Spermatozoal Function Using Dual Fluorescent Staining and Flow Cytometric Analyses, Department of Animal Science, Cornell University, Ithaca, NY, May 13, 1985.
30. Sperm Sexing: New Approaches and Evaluation Techniques, Department of Obstetrics and Gynecology, Texas Tech University Health Science Center, Amarillo, TX, May 13, 1986.
31. Sperm Sexing: New Approaches and Evaluation Techniques, Reproductive and Endocrine Sciences Research Group, Michigan State University East Lansing, MI, February 5, 1987.

32. Bull Semen Evaluation, Bull Fertility Workshop, School of Veterinary Medicine, University of Nevada, Reno, NV, March 14, 1988.
33. Flow Cytometric Analyses of Functional Parameters of Sperm, Departments of Animal Science and Biochemistry/Bophysics, University of California, Davis, CA, February 2, 1990.
34. Fertility Estimation: Seminal and Behavioral Aspects. Faculty Development, Agricultural Profitability Retreat, Nevada Cooperative Extension, Gund Ranch, Austin, NV, December 5, 1990.
35. Stallion Fertility: Physical and Seminal Aspects. Horsemen's Update: Equine Reproduction, Nevada Cooperative Extension, Las Vegas, NV, January 13, 1991.
36. Breeding soundness examination: Cellular aspects, new technologies and semen sexing. California and Nevada Veterinary Medical Association Joint Scientific Seminar and Exposition, Reno, NV, October 26, 1991.